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## A METHOD FOR THE BACTERIOLOGICAL STANDARDIZATION OF DISINFECTANTS.*†

JOHN F. ANDERSON,

Director Hygienic Laboratory, U.S. Public Health and Marine Hospital Service,

AND

THOMAS B. MCCLINTIC,

Passed Assistant Surgeon, U.S. Public Health and Marine Hospital Service.

### Part I.

### INTRODUCTION.

There is no question but that great fraud is practiced in the exploitation of many of the so-called disinfectants that are offered for sale upon the market and are constantly being used by the credulous public. Although the germ theory of disease has long since become an established fact, the average layman either has only a vague conception of it or looks upon it with doubt and suspicion. This skepticism as to the nature of infectious diseases, and the ignorance as to the value of the agents and methods at our command for destroying infection, offer to those manufacturers of disinfectants who care to practice it a fruitful field for deception. Even physicians and health officers are frequently imposed upon by the manufacturers or vendors of so-called disinfectants or germicides.

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It is often too true that the deodorizing powers of these preparations are taken as an index of their germicidal efficiency which, in thus giving a false sense of security, may lead to disastrous results.

As an example, the sale of certain "chlorides" may be cited, the claims for the disinfecting power of which are extravagantly set forth on the label of the container but which, by laboratory examination, are shown to be almost devoid of disinfecting properties, even when used in undiluted strength and under the favorable conditions of a laboratory experiment. Other preparations are sold with the claim of a carbolic coefficient, which often is excessive and cannot be confirmed in other laboratories.

With the methods now customarily used in determining the value of a disinfectant in terms of its carbolic acid coefficient the results that may be obtained even by the same worker are misleading and subject to wide variations.

Therefore the necessity for a satisfactory method for the standardization of the large number of disinfectants offered for sale upon the market becomes evident, and particularly so to all those engaged in public health work.

The Rideal-Walker method[1] is now extensively used, but it is not without its faults. The Lancet method,[2] while not as simple or as easily performed as the Rideal-Walker method, seems to be the best one so far proposed.

Briefly stated, the carbolic acid coefficient in the Rideal-Walker method is arrived at by dividing the figure indicating the degree of dilution of the disinfectant that kills an organism in a given time by that expressing the degree of dilution of the carbolic acid that kills the same organism in the same time under exactly similar conditions. Leaving out details, the determination of the Rideal-Walker coefficient is substantially as follows:

Certain standard conditions are considered essential to the proper performance of the test. Phenol solutions of known strength are used; cultures are grown in a standard medium, transplants being made every 24 hours; the loops used for all inoculations are

[1] S. Rideal, and J. S. A. Walker, *Jour. Roy. San. Inst.*, London, 1903, 24, p. 424.

[2] "The Standardization of Disinfectants [unsigned]," *Lancet*, London, Vol. 177, Nos. 4498, 4499, and 4500.

of a standard size (about 4 mm. in diameter). Usually four dilutions of suitable strengths of the disinfectant to be used are made. Phenol controls of a suitable strength are also prepared. Five c.c. of each of these dilutions are placed in sterile test-tubes to which are added at intervals of one half-minute a 24-hour broth culture of *B. typhosus* in the proportion of 1 drop of culture to each cubic centimeter of disinfectant used (according to Partridge, one drop of culture equals about 0.1 c.c.).

At the end of two and a half minutes a loopful of each of the mixtures is inoculated into a test-tube containing 5 c.c. of standard broth, an interval of half a minute being thus allowed between taking the samples from the different dilutions. This is repeated at 5, 7½, 10, 12½, and 15 minutes. The broth tubes, after being incubated at 37° C. for 48 hours, are examined for growth.

The results of the examination are then noted, and if suitable comparative strengths of the disinfectant and carbolic acid have been selected the carbolic acid coefficient is determined as above stated.

The following table (Table 1) illustrates the manner of determining the carbolic acid coefficient of a disinfectant according to the Rideal-Walker method:

TABLE 1.

*Name*, "A." *Date*, May 18, 1910.
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*, None.
*Subculture Media*, Standard extract broth. *Reaction*, +1.5. *Quantity in Each Tube*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:90 | + | − | − | − | | | | |
| | 1:100 | + | + | + | − | − | − | | |
| Disinfectant "A"... | 1:500 | + | + | − | − | − | − | | |
| | 1:550 | + | + | + | − | − | − | | |
| | 1:600 | + | + | + | + | − | − | | |
| | | | | | | | | 100)550 | |
| | | | | | | | | 5.5 coefficient | |

Early in our work it was realized that, on account of certain faults to be pointed out later, the Rideal-Walker method was not entirely satisfactory for determining the relative values of dis-

infectants in terms of pure phenol. When used under standard conditions as to temperature, organism, media, etc., *approximately* constant results may be obtained by different workers familiar with the technic; but a certain amount of dexterity which can only be obtained by practice is always necessary for carrying out the test.

The great objection to the method, however, and it is almost a vital one, is the latitude allowed in determining the coefficient. This point is strikingly brought out in the following tables (2, 3, and 4):

TABLE 2.

*Date*, May 18, 1910.

*Name*, "A."
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*, None.
*Subculture Media*, Standard extract broth. *Reaction*, +1.5. *Quantity in Each Tube*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:80 | − | − | − | | | | | |
| | 1:90 | + | − | − | − | | | | |
| Disinfectant "A"... | 1:375 | − | − | − | | | | | |
| | 1:400 | + | − | − | − | | | | |
| | 1:425 | + | + | − | − | − | − | | |
| | | | | | | | | 90)400 | |
| | | | | | | | | 4.44 coefficient | |

TABLE 3.

*Date*, May 18, 1910.

*Name*, "A."
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*, None.
*Subculture Media*, Standard extract broth. *Reaction*, +1.5. *Quantity* in *Each Tube*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:90 | + | − | − | − | | | | |
| | 1:100 | + | + | + | − | − | − | | |
| Disinfectant "A"... | 1:450 | + | + | − | − | − | − | | |
| | 1:550 | + | + | + | − | − | − | | |
| | 1:600 | + | + | + | + | − | − | | |
| | | | | | | | | 100)550 | |
| | | | | | | | | 5.5 coefficient | |

TABLE 4.

*Name*, "A." *Date*, May 18, 1910.
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*, None.
*Subculture Media*, Standard extract broth. *Reaction*, +1.5. *Quantity in Each Tube*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:100 | + | + | + | − | − | − | | |
| | 1:110 | + | + | + | + | + | − | | |
| Disinfectant "A"... | 1:600 | + | + | + | + | − | − | | |
| | 1:650 | + | + | + | + | + | − | | |
| | 1:700 | + | + | + | + | + | + | | |
| | | | | | | | | 110)650 | |
| | | | | | | | | 5.91 coefficient | |

Tables 2, 3, and 4 give the determination of the carbolic coefficient for the same disinfectant under exactly similar conditions, the experiments being done on the same day.

In Table 2 the coefficient is 4.44; in Table 3 it is 5.5; in Table 4 it is 5.91. It will be seen, therefore, that according to the bias of the operator a coefficient may be obtained for the same disinfectant varying from 4.44 to 5.91, or a difference of 33.2 per cent—truly a wide variation.

With practice and by selecting for use certain strengths of the disinfectant and carbolic acid, the operator can regulate to a certain extent the time period at which the comparison will be made in determining the coefficient. Furthermore, if more than one strength of the carbolic acid control is used and the results show that more than one of the time periods will admit of comparison, the operator can arbitrarily select the one that will most advantageously suit the purpose of the experiment. Herein lie the principal objections to the Rideal-Walker method of determining the coefficient of disinfectants.

Other minor objections to the method that may be noted are lack of definiteness in the proportion of culture added to the disinfectant, a "drop" being a variable quantity; the latitude allowed in temperature, 18° to 20° C., being a rather wide variation (see results of our experiments at temperatures from 15° to 25° C.); the use of seeding tubes 5 inches in length by $\frac{5}{8}$ inch in diameter which, unless the tubes are handled, offer some difficulty in taking

plants therefrom, and if the tubes are removed for this purpose erroneous results may be obtained by shaking from the sides of the tube organisms that have not been fully exposed to the action of the disinfectant.

The coefficient, as determined by the Lancet Commission, is arrived at as follows: The figure representing the percentage strength of the weakest killing dilution of the phenol is divided by the figure representing the percentage strength of the weakest killing dilution of the unknown disinfectant, both at $2\frac{1}{2}$ and at 30 minutes, the mean resulting figure being taken as the true coefficient. An example of the determination of the carbolic acid coefficient by the Lancet method may be seen from the following table:

TABLE 5.*

DISINFECTANT X.

| MINUTES | DILUTIONS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1–300 | 1–400 | 1–500 | 1–600 | 1–700 | 1–800 | 1–900 | 1–1000 |
| | % 0.333 | % 0.250 | % 0.200 | % 0.166 | % 0.143 | % 0.125 | % 0.111 | % 0.100 |
| 2½ | o | o | + | + | + | | | |
| 5 | | o | o | + | + | + | | |
| 7½ | | o | o | + | + | + | | |
| 10 | | | o | + | + | + | + | |
| 12½ | | | o | + | + | + | + | |
| 15 | | | | + | + | + | + | + |
| 20 | | | | o | + | + | + | + |
| 25 | | | | o | + | + | + | + |
| 30 | | | | o | + | + | + | + |

CARBOLIC ACID CONTROL.

| MINUTES | PERCENTAGE DILUTIONS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1.10 | 1.00 | 0.917 | 0.846 | 0.786 | 0.733 | 0.687 | 0.647 |
| 2½ | o | o | + | + | | | | |
| 5 | o | o | + | + | | | | |
| 25 | | | | | o | o | + | + |
| 30 | | | | | o | o | + | + |

Room temperature 67° F.; + signifies growth; o signifies no growth; blank spaces signify not tested.

The coefficient is therefore:

$$\frac{\frac{1.00}{0.25}+\frac{0.733}{0.166}}{2}=\frac{4.0+4.4}{2}=4.2.$$

* Taken from the report of the Lancet Commission.

The important modifications of the Lancet method on the Rideal-Walker are in the increased number of dilutions employed, sometimes as many as twelve tubes being inoculated in each $2\frac{1}{2}$ minute interval; extension of the number of time intervals to 30 instead of 15 minutes; the use of *B. coli* and MacConkey's bile salt media for subcultures instead of *B. typhosus* and standard extract broth; the amount of the mixture of culture and disinfectant transferred to the subculture tubes; the method of determining the coefficient.

Other minor modifications are the use of special spoons for transplanting instead of loops; the use of "spoonfuls" of the culture for seeding instead of "drops"; a special apparatus for flaming the spoons; and the use of specimen pots instead of test-tubes to contain the mixture of disinfectant and culture.

The Lancet method, on the whole, seems to be a distinct advance over the Rideal-Walker; but nevertheless it appears to have certain rather serious faults.

The Lancet method requires the use of *B. coli* and MacConkey's bile salt media, and their use was proposed for the following claimed advantages over the *B. typhosus* and standard extract broth: (1) It is nonpathogenic; (2) a culture of very constant biological characters may be obtained by carrying on a culture every 24 hours in broth of the same standard composition; and (3) if MacConkey's bile salt media are used for subcultures the risk of misleading results from accidental contamination is practically eliminated.

These claimed advantages do not appear to us to be altogether well founded, for the following reasons: It is well known that there is a greater variability in the different strains of *B. coli communis* than in the various strains of *B. typhosus* and therefore there will be less variation in the organism used in the different laboratories if the latter be used than if *B. coli* is used. The use of a special medium which, by its color reactions, will prevent falsification of results does not greatly appeal to us; if the typhoid bacillus is used and an error is suspected it can be easily cleared up with antityphoid serum. Moreover, besides being comparatively expensive, bile salt media have a much greater restraining influence on

attenuated organisms of the typhoid-colon group, such as is the case after they have been exposed to the action of disinfectants, than is the case with extract broth, the reaction of which is +1.5.

Furthermore, with the Lancet method there seems to be no rule or standard as to the rate of decrease in strengths of the dilutions of disinfectants to be tested. It is evident, for instance, that different results will be obtained if one worker uses dilutions, say of 1:300, 1:350, 1:400, etc., and another uses 1:300, 1:325, 1:350, etc.

Aside from the use of the colon bacillus as a test organism, the special media for subcultures, and the lack of a standard scale for making dilutions, the Lancet method has but little to be objected to. The lengthening of the time for the performance of the test to 30 minutes does not seem to have any very special advantage. The special apparatus required, particularly the "spoons," interfere to some extent with the general employment of the method.

However, notwithstanding the objections noted, we believe the Lancet method to be a distinct improvement over any other that has been proposed up to this time; and that with it constant results can be obtained by different workers.

After doing considerable work with both the Rideal-Walker and the Lancet methods we came to the conclusion that, on account of the great variation in results that may be obtained with the former method, it was not a method to advise for the examination of disinfectants.

While consistent results can be obtained with the Lancet method, we believe, on account of the objections we have stated in the discussion of that method, that this method can be so modified as to greatly increase its usefulness. With this object in view we have taken up in detail each step and factor concerned in the successful carrying out of the test and propose the following method, to be described later, for the determination of the germicidal efficiency of disinfectants as compared with pure phenol.

In proposing this method we desire to make full acknowledgment of our use of the Rideal-Walker and the Lancet methods, especially the latter, as a basis for our work.

## Part II.

### PRINCIPAL FACTORS INVOLVED IN THE EXAMINATION OF DISINFECTANTS.

Lack of attention to the different factors concerned in the examination of disinfectants is responsible for a large percentage of the inconsistencies or discrepancies in results obtained by the same or by different workers when working with the same disinfectant. Unless strict attention is paid to the various influences involved it is useless to expect to find any method satisfactory.

In order the better to emphasize the effect of these influences upon the results obtained the various factors involved will be discussed under the appropriate headings.

#### TEST ORGANISM.

Unless different observers use the same species of organism there can be no possibility of uniformity in results. The coefficient obtained with different species may vary as much as 300 per cent. For this reason it is important that one species be selected for use as the test organism. It would be highly desirable if the same strain of this species could be used by all workers in the testing of disinfectants, as there is often a variation in the resistance of different strains of the same species. This objection does not apply as much to the typhoid organism as to the colon bacillus, and to some other bacteria.

We made a number of comparative tests with different strains of *B. typhosus* and *B. coli*, and found a very much greater difference in the resistance of different strains of the colon bacillus than of the typhoid bacillus.

It is most important that, before being used for a test, the organism be carried over on broth daily for at least one week. In all cases a 24-hour culture should be used, as there is a decided difference in the resistance of a 24-hour and a 48-hour culture, the latter being the more resistant.

In order to avoid clumps in the culture, the 24-hour broth culture should be well shaken and then filtered through sterile filter paper into a sterile test-tube. After this it should be placed

in the water bath in order that it may reach the standard temperature before being added to the disinfectant dilutions.

### TEMPERATURE OF EXPERIMENT.

It is a well-known principle in the use of disinfectants that, within certain limits, the higher the temperature at which the disinfectant is used the greater are its germicidal properties. This increase in the germicidal properties of disinfectants through the influence of heat is not the same for all disinfectants; some, such as formaldehyde, are more strongly influenced than others. The following tables (6 to 9) show the influence of heat upon solutions of phenol and disinfectant "A." It will be seen that, at 15° C., phenol, in a dilution of 1:80, killed the typhoid organism in $2\frac{1}{2}$ minutes, while at a temperature of 30° C. the organism was killed in the same length of time by a dilution of 1:120.

On account of the great variation of temperature in the United States, especially during the summer, it becomes necessary that a standard temperature be adopted. We have adopted a temperature of 20° C. and have devised a simple water bath to be used for maintaining this temperature. This bath consists of a wooden box 20 inches deep, 21 inches long, and 21 inches wide. Inside this box a 14-quart agate-ware pail 10 inches deep is placed and sawdust is well packed around, sufficient being placed on the bottom of the box to bring the rim of the pail on a level with the top of the box.

A tightly fitting wooden cover is placed over the pail, so made that the edges project slightly over the rim. In the cover are a sufficient number of holes for the seeding tubes, a thermometer, and the tube containing the culture. About three inches below the rim of the pail a false bottom of wire gauze is placed; this is for the seeding tubes, etc., to rest on. Water is placed in the pail to within half an inch of the top.

When an experiment is to be made the temperature of the water in the pail is taken, and if above or below 20° C. it is brought to the desired temperature by the addition of either cold or hot water. It will be found that only very slight change takes place in the temperature of the bath in an hour and that it is an easy

matter to keep the temperature at the figure desired. It is of advantage, in regulating the temperature of the bath, to have a spigot in the bottom of the pail to draw off the water when so desired.

TABLE 6.

*Date*, August 11, 1910.

*Name*, Disinfectant "A" and phenol.
*Temperature of Medication*, 15° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*, None.
*Subculture Media*, Extract broth. *Reaction*, +1.5. *Quantity*, 10 c.c

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:70 | − | − | − | | | | | |
| | 1:80 | − | − | − | − | | | | |
| | 1:90 | + | − | − | − | − | − | | |
| | 1:100 | + | + | − | − | − | − | | |
| | 1:110 | + | + | + | + | + | + | | |
| | 1:120 | + | + | + | + | + | + | | |
| Disinfectant "A"... | 1:350 | − | − | − | | | | | |
| | 1:375 | + | − | − | − | − | | | |
| | 1:400 | + | − | − | − | − | − | | |
| | 1:450 | + | + | − | − | − | − | | |
| | 1:500 | + | + | + | − | − | − | | |
| | 1:550 | + | + | + | + | − | − | | |
| | 1:600 | + | + | + | + | + | + | | |
| | 1:650 | + | + | + | + | + | + | | |
| | 1:700 | + | + | + | + | + | + | | |

TABLE 7.

*Date*, August 11, 1910.

*Name*, Disinfectant "A" and phenol.
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*,
*Subculture Media*, Extract broth. *Reaction*, +1.5. *Quantity*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:90 | − | − | − | | | | | |
| | 1:100 | + | − | − | − | − | − | | |
| | 1:110 | + | + | + | + | − | − | | |
| | 1:120 | + | + | + | + | + | + | | |
| Disinfectant "A"... | 1:350 | − | − | − | | | | | |
| | 1:375 | − | − | − | − | | | | |
| | 1:400 | − | − | − | − | − | | | |
| | 1:450 | + | − | − | − | − | − | | |
| | 1:500 | + | − | − | − | − | − | | |
| | 1:550 | + | + | − | − | − | − | | |
| | 1:600 | + | + | − | − | − | − | | |
| | 1:650 | + | + | + | + | + | + | | |
| | 1:700 | + | + | + | + | + | + | | |
| | 1:750 | + | + | + | + | + | + | | |

TABLE 8.

*Name*, Disinfectant "A" and phenol. *Date*, August 11, 1910.
*Temperature of Medication*, 25° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*,
*Subculture Media*, Extract broth. *Reaction*, +1.5. *Quantity*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:100 | − | − | − | | | | | |
| | 1:110 | − | − | − | − | − | | | |
| | 1:120 | + | − | − | − | − | − | | |
| | 1:130 | + | + | + | − | − | − | | |
| | 1:140 | + | + | + | + | + | − | | |
| | 1:150 | + | + | + | + | + | + | | |
| Disinfectant "A"... | 1:450 | − | − | − | − | | | | |
| | 1:500 | − | − | − | − | − | | | |
| | 1:550 | − | − | − | − | − | − | | |
| | 1:600 | − | − | − | − | − | − | | |
| | 1:650 | + | + | − | − | − | − | | |
| | 1:700 | + | + | + | − | − | − | | |
| | 1:750 | + | + | + | − | − | − | | |
| | 1:800 | + | + | + | + | + | + | | |

TABLE 9.

*Name*, Disinfectant "A" and phenol. *Date*, ————, 1910.
*Temperature of Medication*, 30° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*,
*Subculture Media*, Extract broth. *Reaction*, +1.5. *Quantity*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:110 | − | − | − | − | | | | |
| | 1:120 | − | − | − | − | | | | |
| | 1:130 | + | − | − | − | − | − | | |
| | 1:140 | + | + | − | − | − | − | | |
| | 1:150 | + | + | + | − | − | − | | |
| | 1:500 | − | − | − | − | | | | |
| | 1:550 | − | − | − | − | | | | |
| | 1:600 | − | − | − | − | − | − | | |
| | 1:650 | − | − | − | − | − | − | | |
| | 1:700 | − | − | − | − | − | − | | |
| | 1:750 | + | − | − | − | − | − | | |
| | 1:800 | + | + | + | − | − | − | | |
| | 1:850 | + | + | + | + | + | + | | |
| | 1:900 | + | + | + | + | + | + | | |

## PROPORTION OF CULTURE TO DISINFECTANT.

As disinfection is the result of the chemical action of the disinfecting agent upon the test organism, mass action is an important factor in the testing of disinfectants. By this is meant that within certain limits the greater the number of bacteria added to the disinfectant dilution the stronger the dilution required to do the same work. For this reason it is important that the amount of

culture to be added to the dilution should be stated in definite quantities and not in "drops" or in "spoonfuls." We have adopted the practice of using 0.1 c.c. of a 24-hour broth culture. For measuring this we use a delivery pipette graduated in tenths.

The influence of different amounts of culture is shown in Tables 10 and 11.

TABLE 10.

*Name*, "B." *Date*, May 10, 1910.
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., broth culture, filtered.
*Proportion of Culture and Disinfectant*, 0.5 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*,
*Subculture Media*, Extract broth. *Reaction*, +1.5. *Quantity*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:70 | − | − | | | | | | |
| | 1:80 | − | − | − | − | − | | 80)900 | |
| | 1:90 | + | − | − | − | − | − | | 11.25 |
| | 1:100 | + | + | + | + | + | − | 100)1200 | |
| | 1:110 | + | + | + | + | + | + | | 12.00 |
| | 1:120 | + | + | + | + | + | + | | 2)23.25 |
| | | | | | | | | | 11.62 |
| Disinfectant "B"... | 1:800 | − | − | − | | | | | |
| | 1:900 | − | − | − | − | | | | |
| | 1:1000 | + | − | − | − | − | − | | |
| | 1:1100 | + | + | + | − | − | − | | |
| | 1:1200 | + | + | + | + | + | − | | |
| | 1:1300 | + | + | + | + | + | + | | |
| | 1:1400 | + | + | + | + | + | + | | |
| | 1:1500 | + | + | + | + | + | + | | |

TABLE 11.

*Name*, "B." *Date*, May 16, 1910.
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*,
*Subculture Media*, Extract broth. *Reaction*, +1.5. *Quantity*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:80 | − | − | − | | | | | |
| | 1:90 | + | − | − | − | − | | 80) 1300 | |
| | 1:100 | + | + | − | − | − | − | | 16.25 |
| | 1:110 | + | + | + | + | + | + | 100) 1500 | |
| | 1:120 | + | + | + | + | + | + | | 15.00 |
| | | | | | | | | | 2)31.25 |
| Disinfectant "B"... | 1:1100 | − | − | − | | | | | 15.62 |
| | 1:1200 | − | − | − | − | | | | |
| | 1:1300 | − | − | − | − | − | − | | |
| | 1:1400 | + | − | − | − | − | − | | |
| | 1:1500 | + | + | + | − | − | − | | |
| | 1:1600 | + | + | + | + | + | + | | |

AMOUNT OF INOCULATION OF SUBCULTURE TUBES.

The amount of the mixture of culture and disinfectant transferred to the subculture tubes for observation as to the effect of the disinfectant upon the test organism has an important influence upon the result of the experiment. If in doing two experiments the subculture inoculations are made, one with loops and the other with spoons which carry over considerably more than the loops, it will be observed that a growth of the organism is often obtained after exposure to slightly stronger solutions of the disinfectant when the spoons are used than is the case with the loops. While this is not very marked, still the use of the spoon will give a slightly lower coefficient than the loop and it is therefore important that a standard-size loop be used for the inoculation of the subculture tubes, since the cost and the difficulty of procuring the spoons make them more or less impracticable. A large number of experiments were done both with loops and spoons, and Tables 12 and 13 show about the average difference in results:

TABLE 12.

*Name*, "B." *Date*, May 13, 1900.
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*,
*Subculture Media*, Standard broth. *Reaction*, +1.5. *Quantity*, 10 c.c.
Inoculations made with loops holding about 0.016 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol..... | 1:80 | − | − | − | | | | | |
| | 1:90 | + | − | − | − | − | − | | |
| | 1:100 | + | + | + | + | − | − | | |
| | 1:110 | + | + | + | + | + | + | 80) 1300 | |
| | 1:120 | + | + | + | + | + | + | | 16.25 |
| Disinfectant "B"... | 1:1100 | − | − | − | | | | 100) 1600 | |
| | 1:1200 | − | − | − | | | | | 16.00 |
| | 1:1300 | − | − | − | − | | | | 2)32.25 |
| | 1:1400 | + | − | − | − | − | − | | 16.12 |
| | 1:1500 | + | − | − | − | − | − | | |
| | 1:1600 | + | + | + | − | − | − | | |
| | 1:1700 | + | + | + | + | + | + | | |
| | 1:1800 | + | + | + | + | + | + | | |

TABLE 13.

*Date*, May 11, 1910.

*Name*, "B."
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*,
*Subculture Media*, Standard broth. *Reaction*, +1.5. *Quantity*, 10 c.c.
Inoculations made with spoon holding about 0.16 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | | |
| Phenol | 1:80 | − | − | − | − | | | 80) 1200 | |
| | 1:90 | + | − | − | − | − | − | | 15.00 |
| | 1:100 | + | + | − | − | − | − | 100) 1500 | |
| | 1:110 | + | + | + | + | + | + | | 15.00 |
| | 1:120 | + | + | + | + | + | + | | 2)30.00 |
| Hyco | 1:1100 | − | − | − | − | | | | 15.00 |
| | 1:1200 | − | − | − | − | − | | | |
| | 1:1300 | + | − | − | − | − | − | | |
| | 1:1400 | + | + | − | − | − | − | | |
| | 1:1500 | + | + | + | − | − | − | | |
| | 1:1600 | + | + | + | + | + | + | | |
| | 1:1700 | + | + | + | + | + | + | | |

MEDIA FOR SUBCULTURES.

There is probably no one factor, with the possible exception of temperature, that has more to do with irregularities in results than the media used for subcultures. Where the typhoid bacillus is used for the test organism, as in the Rideal-Walker method, and the method proposed by us, it is of paramount importance that the media have a reaction of just +1.5. A reaction greater or less than this exerts a decided inhibiting action upon the growth of the transplanted organism. This is an important point, for if the transplant is made from a test dilution which is just under the killing strength of the disinfectant, the inhibiting action of the media may be sufficient to prevent growth, thus giving a false result. In the hands of different workers a difference in the reaction of the media may result from the degree to which the color reaction in titration is carried. We always carry it to the point where the pink color is distinctly perceptible, but even then there seems to be at times a slight difference in various lots of our media.

The following experiments (Table 14) will show the marked effect of the difference in reaction of the subculture media, all the other conditions of the experiments being identical.

TABLE 14.

*Date*, August 4, 1910.

*Name*, Phenol.
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., in standard broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.

| Reaction of Media to Phenolphthalein | Dilution of Phenol | Time Culture Was Exposed to Action of Phenol, in Minutes: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 |
| Neutral | 1:100 | − | − | − | − | | |
| | 1:110 | − | − | − | − | − | |
| | 1:120 | − | − | − | − | − | − |
| | 1:130 | + | − | − | − | − | − |
| | 1:140 | + | + | + | − | − | − |
| | 1:150 | + | + | + | + | + | − |
| +0.5 | 1:100 | − | − | − | | | |
| | 1:110 | − | − | − | − | | |
| | 1:120 | + | − | − | − | − | − |
| | 1:130 | + | + | − | − | − | − |
| | 1:140 | + | + | + | + | − | − |
| | 1:150 | + | + | + | + | + | − |
| +1.0 | 1:100 | − | − | − | − | | |
| | 1:110 | + | − | − | − | − | − |
| | 1:120 | + | + | + | − | − | − |
| | 1:130 | + | + | + | + | + | + |
| | 1:140 | + | + | + | + | + | + |
| +1.5 (standard) | 1:90 | − | − | − | | | |
| | 1:100 | + | − | − | − | − | − |
| | 1:110 | + | + | + | − | − | − |
| | 1:120 | + | + | + | + | + | + |
| | 1:130 | + | + | + | + | + | + |
| +2.0 | 1:90 | − | − | | | | |
| | 1:100 | − | − | − | − | − | − |
| | 1:110 | + | − | − | − | − | − |
| | 1:120 | + | + | + | − | − | − |
| | 1:130 | + | + | + | + | + | + |
| | 1:140 | + | + | + | + | + | + |

It is a noteworthy fact that the influence of the reaction of the subculture media upon the growth of the exposed organism was decidedly more pronounced after it had been exposed to phenol than to any of the other disinfectants tried.

It was found too that a more vigorous growth and a growth from stronger solutions were obtained when the exposed organisms were planted in meat broth than when they were planted in extract broth. It is therefore evident from the above that the reaction and character of the subculture media has an important bearing upon the results obtained in determining the phenol coefficient of disinfectants. However, as extract broth is more uniform in composition, more easily prepared, and cheaper than meat broth, we recommend that extract broth be always used, and when it is not, that the fact be so stated.

The amount of media in the tubes for subculture should be sufficient to prevent any antiseptic action, due to the transferred disinfectant. With substances such as chinosol and bichloride of mercury, this is often an important point.

It may be stated here that in our work with some disinfectants, particularly those containing coal-tar products, the disinfectant carried over in making the inoculations of the subcultures caused a distinct cloudiness in the media; but after 48 hours' incubation, this always cleared up so that there was no difficulty in making out the presence or absence of growth.

MacConkey's bile salt medium was given a limited trial with *B. coli communis*. We found that after exposing the *B. coli communis* to the action of a 1 per cent solution of carbolic acid and planting in MacConkey's medium and extract broth respectively every $2\frac{1}{2}$ minutes for 15 minutes, and incubating for 48 hours, there was a growth in all the tubes of the extract broth, but only in the $2\frac{1}{2}$ minutes' tube of MacConkey's medium. This condition or result was more marked with carbolic acid than with any other disinfectant tried.

When using the *B. typhosus* the possibility of contamination in the tubes of broth that show a growth at the end of 48 hours can be determined by the use of antityphoid serum.

### ORGANIC MATTER.

We have said nothing as to the introduction of organic matter when making the tests, although we have made many experiments as to the influence of this factor in the examination of disinfectants. We have considered very carefully the advisability of the addition of some form of organic matter, but finally decided not to include it in the method proposed by us. After all, the comparative efficiency of a disinfectant can be made as well without as with organic matter, even though it may be claimed that in the practical use of the substances organic matter be present.

As there are certain disinfectants, such as bichloride of mercury, which are very adversely influenced by organic matter and others not at all—or favorably—affected, it does not seem to us wise to require the presence of organic matter, except where information

on this point is especially desired. It would be of value if the coefficient be determined both with and without the addition of organic matter. When organic matter is to be added we have preferred to use it in the form of a killed (by heat) 24-hour broth culture of the typhoid bacillus.

## Part III.

### HYGIENIC LABORATORY PHENOL COEFFICIENT.

Having discussed the necessity for a satisfactory method of standardizing disinfectants and the factors involved in the examination of disinfectants, we present below the method we have devised.

When this method is used for the standardization of disinfectants we recommend that it be referred to as the "Hygienic Laboratory Phenol Coefficient."

We prefer to use the word "phenol" instead of "carbolic acid" when speaking of the coefficient, especially since certain dealers advertise for sale carbolic acids which vary greatly in the proportion of phenol present.

#### MEDIA.

Standard extract broth is used, both for the culture to be tested and for the subcultures made after exposure to the disinfectant. The broth is made from Liebig's extract of beef and is in exact accordance with the standard methods adopted by the American Public Health Association for water analysis. Ten c.c. of the broth are put into each test-tube. This amount of broth has been found sufficient to avoid any antiseptic action of the disinfectant carried over. It is important that the reaction of the media is just $+1.5$.

#### ORGANISM.

For the test organism a 24-hour-old broth culture in extract broth of the *B. typhosus* (Hopkins) is used. Before beginning a test the culture should be carried over every 24 hours on at least three successive days. For carrying over the culture one loopful of a 4 mm. platinum loop is used.

Before being added to the disinfectant the culture is well shaken, filtered through sterile filter paper, and placed in the water

bath in order that it may reach a temperature of 20° C. before being added to the disinfectant.

### TEMPERATURE.

A standard temperature of 20° C. has been adopted for all experiments. This temperature is obtained by the use of a specially devised water bath. The cultures and dilutions of the disinfectant are brought to this temperature before the beginning of the test.

### PROPORTION OF CULTURE TO DISINFECTANT.

One-tenth c.c. of the culture is used, added to 5 c.c. of the disinfectant dilution. The amount of culture is measured with a pipette graduated in tenths of a cubic centimeter.

### INOCULATION LOOPS.

For making the transfer of the culture after exposure to the disinfectant a platinum loop 4 mm. in diameter of 23 U.S. standard gauge wire is used. We have found it is of advantage to have at least 4, and preferably 6, loops. In order to save time in flaming the following method was devised:

A block about 3 inches wide, 10 inches high, and 12 inches long, containing four or six grooves, spaced two inches apart, is used. Into each of the grooves the platinum loop is laid so that the end of the loops extend about 5 inches beyond the side of the block. The first step in the operation is to sterilize each loop by flaming with a fan-tail Bunsen burner before beginning the experiment.

When ready to begin the operation the loop farthest from the operator is taken in the right hand and the inoculation made. It is then replaced in the groove with the right hand and the Bunsen burner (fan-tail) placed under it with the left hand. The next loop is then used, replaced in its groove, and the Bunsen burner placed under it with the left hand, the first loop having been heated to redness while the second loop was in use. This procedure is then continued until all the inoculations have been made. The time required in making the inoculations and in replacing the loop is short, it being found that 15 seconds is ample.

INCUBATION.

The subcultures are incubated 48 hours at 37° C., and the results then read off and tabulated.

DILUTIONS.

Capacity pipettes for the original dilutions are invariably used. For the phenol controls a standard dilution of pure phenol (Merck) is made and standardized by the U.S.P. method (Koppeschaar) to contain exactly 5 per cent of pure phenol by weight. From this stock solution the higher dilutions are made fresh each day for that day's test.

For the dilutions of the disinfectant a 5 per cent solution is made by adding 5 c.c. of the disinfectant to 95 c.c. of sterile distilled water. A standardized 5 c.c. capacity pipette is used for this and after filling the pipette all excess of the disinfectant on the outside of the pipette is wiped off with sterile gauze. The contents of the pipette are then delivered into a cylinder containing 95 c.c. of sterile distilled water and the pipette washed out as clean as possible by aspiration and blowing out the contents of the pipette into the cylinder. The contents of the cylinder are then thoroughly shaken and the dilutions up to 1:500 made from it, using delivery pipettes for measuring. For those disinfectants which do not readily form a 5 per cent solution we make a 1 per cent stock solution and from this make the dilutions greater than 1:100 in accordance with the second table of dilutions. If greater dilutions than 1:500 are to be made, a 1 per cent solution is made from the 5 per cent solution, and the higher dilutions made from this.

We have adopted the following scale for making dilutions:

For dilutions up to 1:70, increase or decrease by a difference of 5 (i.e., 5 parts of water).

| | | |
|---|---|---|
| From 1:70 | to 1:160 | by a difference of 10 |
| From 1:160 | to 1:200 | by a difference of 20 |
| From 1:200 | to 1:400 | by a difference of 25 |
| From 1:400 | to 1:900 | by a difference of 50 |
| From 1:900 | to 1:1800 | by a difference of 100 |
| From 1:1800 | to 1:3200 | by a difference of 200 |

and so on if higher dilutions are necessary.

It is important that the cylinders used for making the dilutions be correctly graduated, as we have found disregard of this factor an important source of error. It is preferable to use standardized cylinders and pipettes, and we recommend that they be used whenever possible. They of course should be perfectly clean. For making the dilutions in accordance with the above scheme we have found the following tables of much service:

TABLE 15 (FOR DILUTIONS).

STOCK 5 PER CENT SOLUTION.

(5 c.c. disinfectant + 95 c.c. distilled water) = Solution A.

| | c.c. of A | c.c. Dist. Water | | c.c. of A | c.c. Dist. Water | | c.c. of A | c.c. Dist. Water |
|---|---|---|---|---|---|---|---|---|
| 1:20 | = 20 | + 0 | or | 10 | + 0 | or | 4 | + 0 |
| 1:25 | = 20 | + 5 | or | 10 | + 2½ | or | 4 | + 1 |
| 1:30 | = 20 | + 10 | or | 10 | + 5 | or | 4 | + 2 |
| 1:35 | = 20 | + 15 | or | 10 | + 7½ | or | 4 | + 3 |
| 1:40 | = 20 | + 20 | or | 10 | + 10 | or | 4 | + 4 |
| 1:45 | = 20 | + 25 | or | 10 | + 12½ | or | 4 | + 5 |
| 1:50 | = 20 | + 30 | or | 10 | + 15 | or | 4 | + 6 |
| 1:55 | = 20 | + 35 | or | 10 | + 17½ | or | 4 | + 7 |
| 1:60 | = 20 | + 40 | or | 10 | + 20 | or | 4 | + 8 |
| 1:65 | = 20 | + 45 | or | 10 | + 22½ | or | 4 | + 9 |
| 1:70 | = 20 | + 50 | or | 10 | + 25 | or | 4 | + 10 |
| 1:70 | = 20 | + 50 | or | 10 | + 25 | or | 4 | + 10 |
| 1:80 | = 20 | + 60 | or | 10 | + 30 | or | 4 | + 12 |
| 1:90 | = 20 | + 70 | or | 10 | + 35 | or | 4 | + 14 |
| 1:100 | = 20 | + 80 | or | 10 | + 40 | or | 4 | + 16 |
| 1:110 | = 20 | + 90 | or | 10 | + 45 | or | 4 | + 18 |
| 1:120 | = 20 | + 100 | or | 10 | + 50 | or | 4 | + 20 |
| 1:130 | = 20 | + 110 | or | 10 | + 55 | or | 4 | + 22 |
| 1:140 | = 20 | + 120 | or | 10 | + 60 | or | 4 | + 24 |
| 1:150 | = 20 | + 130 | or | 10 | + 65 | or | 4 | + 26 |
| 1:160 | = 20 | + 140 | or | 10 | + 70 | or | 4 | + 28 |
| 1:160 | = 20 | + 140 | or | 10 | + 70 | or | 4 | + 28 |
| 1:180 | = 20 | + 160 | or | 10 | + 80 | or | 4 | + 32 |
| 1:200 | = 20 | + 180 | or | 10 | + 90 | or | 4 | + 36 |
| 1:200 | = 20 | + 180 | or | 4 | + 36 | or | 2 | + 18 |
| 1:225 | = 20 | + 205 | or | 4 | + 41 | or | 2 | + 20½ |
| 1:250 | = 20 | + 230 | or | 4 | + 46 | or | 2 | + 23 |
| 1:275 | = 20 | + 255 | or | 4 | + 51 | or | 2 | + 25½ |
| 1:300 | = 20 | + 280 | or | 4 | + 56 | or | 2 | + 28 |
| 1:325 | = 20 | + 305 | or | 4 | + 61 | or | 2 | + 30½ |
| 1:350 | = 20 | + 330 | or | 4 | + 66 | or | 2 | + 33 |
| 1:375 | = 20 | + 355 | or | 4 | + 71 | or | 2 | + 35½ |
| 1:400 | = 20 | + 380 | or | 4 | + 76 | or | 2 | + 38 |
| 1:450 | = 20 | + 430 | or | 4 | + 86 | or | 2 | + 43 |
| 1:500 | = 20 | + 480 | or | 4 | + 96 | or | 2 | + 48 |

TABLE 16 (FOR DILUTIONS).

STOCK 1 PER CENT SOLUTION.

(1 c.c. disinfectant + 99 c.c. distilled water) = Solution A.

| | c.c. of A | c.c. Dist. Water | | c.c. of A | c.c. Dist. Water | | c.c. of A | c.c. Dist. Water |
|---|---|---|---|---|---|---|---|---|
| 1:100 | =100 | + 0 | or | 10 | + 0 | | | |
| 1:110 | =100 | + 10 | or | 10 | + 1 | | | |
| 1:120 | =100 | + 20 | or | 10 | + 2 | | | |
| 1:130 | =100 | + 30 | or | 10 | + 3 | | | |
| 1:140 | =100 | + 40 | or | 10 | + 4 | | | |
| 1:150 | =100 | + 50 | or | 10 | + 5 | | | |
| 1:160 | =100 | + 60 | or | 10 | + 6 | | | |
| 1:160 | =100 | + 60 | or | 10 | + 6 | | | |
| 1:180 | =100 | + 80 | or | 10 | + 8 | | | |
| 1:200 | =100 | + 100 | or | 10 | + 10 | | | |
| 1:200 | =100 | + 100 | or | 10 | + 10 | or | 4 | + 4 |
| 1:225 | =100 | + 125 | or | 10 | + 12½ | or | 4 | + 5 |
| 1:250 | =100 | + 150 | or | 10 | + 15 | or | 4 | + 6 |
| 1:275 | =100 | + 175 | or | 10 | + 17½ | or | 4 | + 7 |
| 1:300 | =100 | + 200 | or | 10 | + 20 | or | 4 | + 8 |
| 1:325 | =100 | + 225 | or | 10 | + 22½ | or | 4 | + 9 |
| 1:350 | =100 | + 250 | or | 10 | + 25 | or | 4 | + 10 |
| 1:375 | =100 | + 275 | or | 10 | + 27½ | or | 4 | + 11 |
| 1:400 | =100 | + 300 | or | 10 | + 30 | or | 4 | + 12 |
| 1:400 | = 10 | + 30 | or | 4 | + 12 | or | 2 | + 6 |
| 1:450 | = 10 | + 35 | or | 4 | + 14 | or | 2 | + 7 |
| 1:500 | = 10 | + 40 | or | 4 | + 16 | or | 2 | + 8 |
| 1:550 | = 10 | + 45 | or | 4 | + 18 | or | 2 | + 9 |
| 1:600 | = 10 | + 50 | or | 4 | + 20 | or | 2 | + 10 |
| 1:650 | = 10 | + 55 | or | 4 | + 22 | or | 2 | + 11 |
| 1:700 | = 10 | + 60 | or | 4 | + 24 | or | 2 | + 12 |
| 1:750 | = 10 | + 65 | or | 4 | + 26 | or | 2 | + 13 |
| 1:800 | = 10 | + 70 | or | 4 | + 28 | or | 2 | + 14 |
| 1:850 | = 10 | + 75 | or | 4 | + 30 | or | 2 | + 15 |
| 1:900 | = 10 | + 80 | or | 4 | + 32 | or | 2 | + 16 |
| 1:900 | = 5 | + 40 | or | 4 | + 32 | or | 2 | + 16 |
| 1:1000 | = 5 | + 45 | or | 4 | + 36 | or | 2 | + 18 |
| 1:1100 | = 5 | + 50 | or | 4 | + 40 | or | 2 | + 20 |
| 1:1200 | = 5 | + 55 | or | 4 | + 44 | or | 2 | + 22 |
| 1:1300 | = 5 | + 60 | or | 4 | + 48 | or | 2 | + 24 |
| 1:1400 | = 5 | + 65 | or | 4 | + 52 | or | 2 | + 26 |
| 1:1500 | = 5 | + 70 | or | 4 | + 56 | or | 2 | + 28 |
| 1:1600 | = 5 | + 75 | or | 4 | + 60 | or | 2 | + 30 |
| 1:1700 | = 5 | + 80 | or | 4 | + 64 | or | 2 | + 32 |
| 1:1800 | = 5 | + 85 | or | 4 | + 68 | or | 2 | + 34 |
| 1:1800 | = 5 | + 85 | or | 4 | + 68 | or | 2 | + 34 |
| 1:2000 | = 5 | + 95 | or | 4 | + 76 | or | 2 | + 38 |
| 1:2200 | = 5 | + 105 | or | 4 | + 84 | or | 2 | + 42 |
| 1:2400 | = 5 | + 115 | or | 4 | + 92 | or | 2 | + 46 |
| 1:2600 | = 5 | + 125 | or | 4 | + 100 | or | 2 | + 50 |
| 1:2800 | = 5 | + 135 | or | 4 | + 108 | or | 2 | + 54 |
| 1:3000 | = 5 | + 145 | or | 4 | + 116 | or | 2 | + 58 |
| 1:3200 | = 5 | + 155 | or | 4 | + 124 | or | 2 | + 62 |

## SEEDING TUBES.

The seeding tubes are glass test-tubes 1 inch in diameter and about 3 inches long, with round bottoms. In order to measure the disinfectant into them they are placed in a suitable wooden stand to receive them. We found it convenient to use a wooden block containing six rows of 15 holes each for the disinfectant to be tested and a separate stand for the phenol controls. The tubes

are placed in the stand and each marked with the strength of dilution it is to contain. The rows of tubes running crosswise represent the same strength dilution, while the rows running lengthwise represent the different strengths to be used in the experiment.

Starting with the lowest dilution (i.e., the strongest), the cylinder is shaken, then 5 c.c. are measured into the tubes of the row to receive that strength, using a 5 c.c. delivery pipette. In order to economize glassware, the same pipette is used for measuring out the next dilution, first blowing out as much of the remaining liquid as possible, then drawing a pipetteful of the next dilution to be used and discarding that; then filling the pipette a second time, which is then emptied into the seeding tube.

The measuring out being completed, the tubes are placed in the water bath and allowed to stand a few minutes in order that the disinfectant solution may reach the standard temperature. We have not found it necessary to use cotton plugs in the seeding tubes. They are sterilized in paper-lined wire baskets, with the closed end of the tubes up.

### SUBCULTURE TUBE-RACKS.

Wooden racks, with five rows of 14 holes each, are used for holding the subculture tubes, and as plants are made from each mixture of culture and disinfectant every $2\frac{1}{2}$ minutes up to 15 minutes six tubes are required for each dilution. Thus, in each rack we have ten rows of six tubes each with two empty cross rows of holes left, which are utilized by placing over in the next row each tube as it is planted. This makes it easy to keep run of the tubes that are planted. It is well also always to plant from the seeding tube in a certain hole in the water bath into a certain row of tubes in the rack. This, after a little practice, will help to avoid errors in planting.

### METHOD OF CONDUCTING THE TEST.

If there are in one experiment more than 10 dilutions of the disinfectant, including the phenol controls, the stronger solutions of the disinfectant and phenol are tested first, as it will not be necessary to plant them after $7\frac{1}{2}$ minutes. The weaker solutions are

then immediately done and are planted every $2\frac{1}{2}$ minutes for 15 minutes.

For keeping the time a stop watch can be used, but an ordinary watch will serve the same purpose by simply starting on the $2\frac{1}{2}$ or 5 minute periods.

When everything is in readiness the culture is added to the disinfectant solutions with a sterile pipette in tenths of a cubic centimeter.

To add the culture, the seeding tube containing the disinfectant is removed from the water bath with the left hand and slanted at an angle of about 45°, and with the right hand the end of the pipette containing the culture is introduced and lightly touched against the side of the tube where the liquid has run away on account of the slanting. At the proper time the culture is allowed to run into the disinfectant solution, the pipette removed, the tube straightened up, gently shaken three times, and replaced in the water bath. The other tubes are done the same way in succession, and it will be found that 15 seconds is ample time for each tube. By adding the culture to the disinfectant with a pipette touched against the side of the seeding tube, accurate measurements can be made and each tube receive exactly the same amount of "seeding," which is not the case when the culture is added by the "drop."

If ten tubes are to be inoculated, only a few seconds will remain after inoculating the last tube before a plant from the first tube will have to be made.

The mixing tubes are not removed or disturbed in making the planting except to insert the loop or spoon into them, touch the bottom, withdraw, and then make the plant in broth. Every effort is made to insert and withdraw the loops and spoons in a uniform manner. The loops and spoons are bent to an angle of about 45° where they are joined on to the shank, and therefore are always filled with the mixture when withdrawn from the seeding tubes. After making the plants the loops or spoons are flamed as already described.

After an experiment is finished the date and any necessary details can be marked on one of the broth tubes and the rack placed in the incubator at 37° C. for 48 hours. At the end of this

time the results are recorded on a chart specially devised for the purpose.

DETERMINING THE COEFFICIENT.

After a large number of experiments we have concluded that the method employed by the Lancet Commission, with certain modifications, is the best one for determining the coefficient, i.e., the mean between the strength and time coefficients.

In performing the test, plants are made every $2\frac{1}{2}$ minutes up to and including 15 minutes. To determine the coefficient, the figure representing the degree of dilution of the weakest strength of the disinfectant that kills within $2\frac{1}{2}$ minutes is divided by the figure representing the degree of dilution of the weakest strength of the phenol control that kills within the same time. The same is done for the weakest strength that kills in 15 minutes. The mean of the two is the coefficient. The method of determining the coefficient will be seen in Table 17.

TABLE 17.

*Name*, "A." *Date*, May 18, 1910
*Temperature of Medication*, 20° C.
*Culture Used*, B. typhosus, 24-hr., extract broth, filtered.
*Proportion of Culture and Disinfectant*, 0.1 c.c.+5 c.c.
*Organic Matter*, None. *Kind*, None. *Amount*, None.
*Subculture Media*, Standard extract broth. *Reaction*, +1.5. *Quantity in Each Tube*, 10 c.c.

| Sample | Dilution | Time Culture Exposed to Action of Disinfectant for Minutes: | | | | | | Phenol Coefficient | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | $2\frac{1}{2}$ | 5 | $7\frac{1}{2}$ | 10 | $12\frac{1}{2}$ | 15 | | |
| Phenol..... | 1:80 | − | − | − | | | | | |
| | 1:90 | + | − | − | − | | | 80)375 | |
| | 1:100 | + | + | + | − | − | − | | 4.69 |
| | 1:110 | + | + | + | + | + | − | 110)650 | |
| | | | | | | | | | 5.91 |
| Disinfectant "A"... | 1:350 | − | − | − | | | | | 2)10.60 |
| | 1:375 | − | − | − | | | | | 5.30= |
| | 1:400 | + | − | − | − | | | | coefficient |
| | 1:425 | + | + | − | − | − | − | | |
| | 1:450 | + | + | − | − | − | − | | |
| | 1:500 | + | + | − | − | − | − | | |
| | 1:550 | + | + | + | − | − | − | | |
| | 1:600 | + | + | + | + | − | − | | |
| | 1:650 | + | + | + | + | + | − | | |
| | 1:700 | + | + | + | + | + | + | | |
| | 1:750 | + | + | + | + | + | + | | |

TO DETERMINE THE COMPARATIVE COST PER UNIT OF EFFICIENCY.

When bids are solicited for supplying disinfectants they should be required to be made so as to show the comparative cost per

100 units of efficiency of the disinfectant as compared with 100 units of pure phenol. It is manifestly cheaper to purchase a disinfectant that sells for 60 cents a gallon than one that sells for 30 cents a gallon if the former has four times the efficiency of the latter.

The true cost of a disinfectant can be determined only by taking into consideration the phenol coefficient and the cost per gallon of the disinfectant.

The following table (18) is a good illustration of the value of a determination of the comparative cost per 100 units of disinfectant in terms of 100 units of pure phenol:

TABLE 18.

| Disinfectant | Phenol Coefficient | Price per Gallon | Relative Cost per 100 Units of Efficiency as Compared with Pure Phenol |
|---|---|---|---|
| Car | 2.12 | $0.30 | 5.2 |
| Chl | 4.44 | 1.00 | 8.4 |
| Phi | 1.40 | 0.37 | 9.9 |
| Cre | 1.13 | 0.44 | 14.5 |
| Nap | 0.44 | 0.41 | 34.8 |
| Zod | 0.25 | 0.40 | 59.6 |
| Pure phenol | 1.00 | 2.67 | 100.0 |

It will be seen that the substance Chl has a higher coefficient than any of the others in the table, but its high cost per gallon results in its being placed second in cost per 100 units.

The cost per 100 units of efficiency as compared with pure phenol is obtained by first dividing the cost per gallon of the disinfectant by the cost per gallon of pure phenol; this gives the price ratio between the disinfectant and pure phenol; the cost ratio is then divided by the phenol coefficient, which gives us the cost per unit of efficiency as compared with pure phenol = 1. The cost per unit is then multiplied by 100 to give the cost per 100 units.

## EXAMINATION OF COMMERCIAL DISINFECTANTS.

NOTE.—We are now engaged in a study of the various proprietary disinfectants found upon the market, using the method which we have described in this paper. We propose to determine, in this study, the phenol coefficient with and without organic matter and the comparative cost per unit efficiency of the disinfectants studied by us.

Our studies have gone far enough to show more plainly than we ever saw before the great fraud that is being practiced upon the American people, health officers and laymen alike, in the exploitation of some so-called "disinfectants."